SUPPORTING INFORMATION

Virus-free Method to Control and Enhance Extracellular Vesicle Cargo Loading and Delivery

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S1

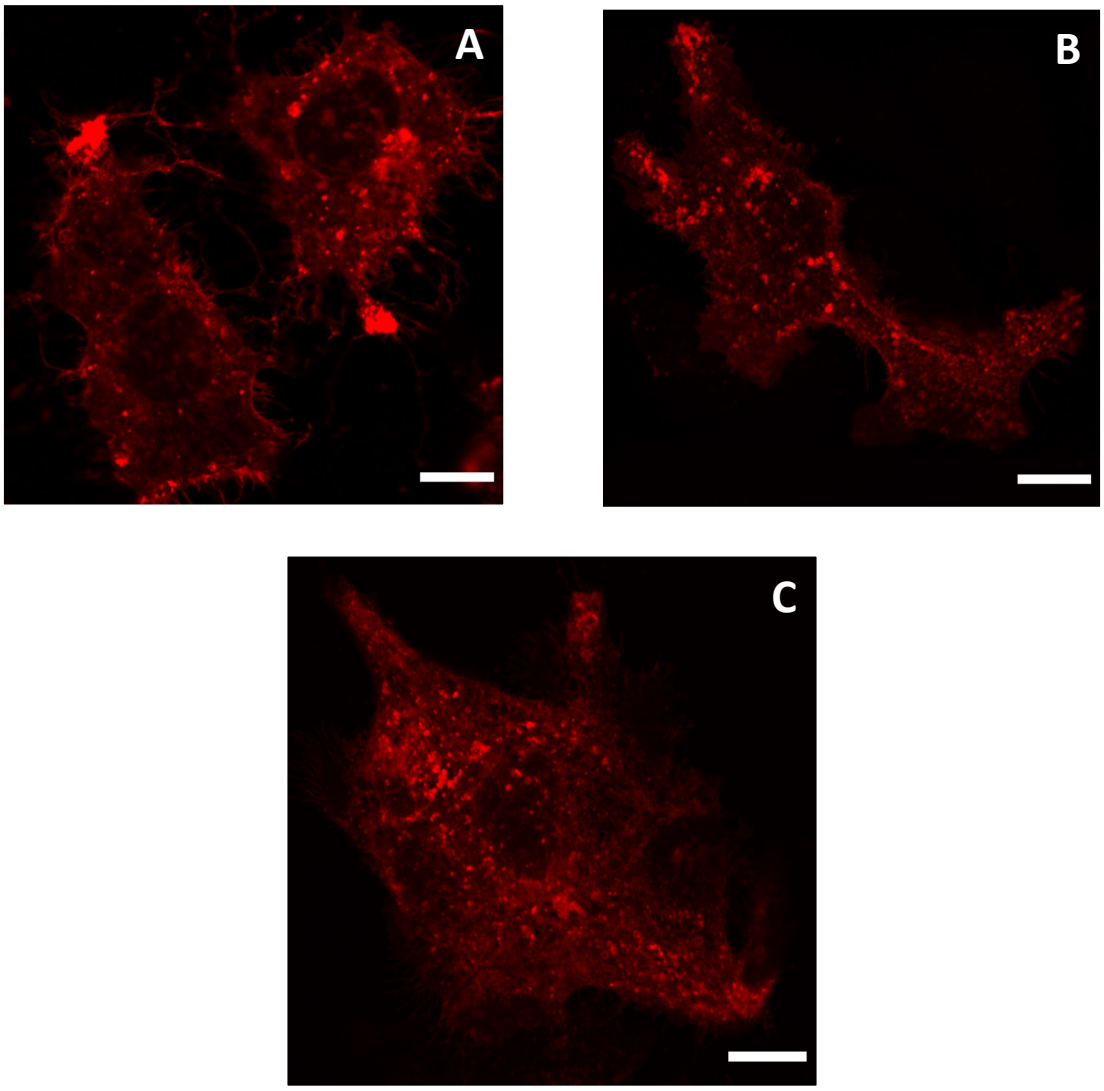

Figure S1. ***FKBP$_2$-RFP-CD63 pattern.*** Confocal imaging of FKBP$_2$-RFP-CD63 (Loader) transiently expressed in (A) HEK293T (embryonic origin), (B) SW480 (colon adenocarcinoma), (C) EGI-1 (cholangiocarcinoma) wild type cells. Scale bar, 10μm.

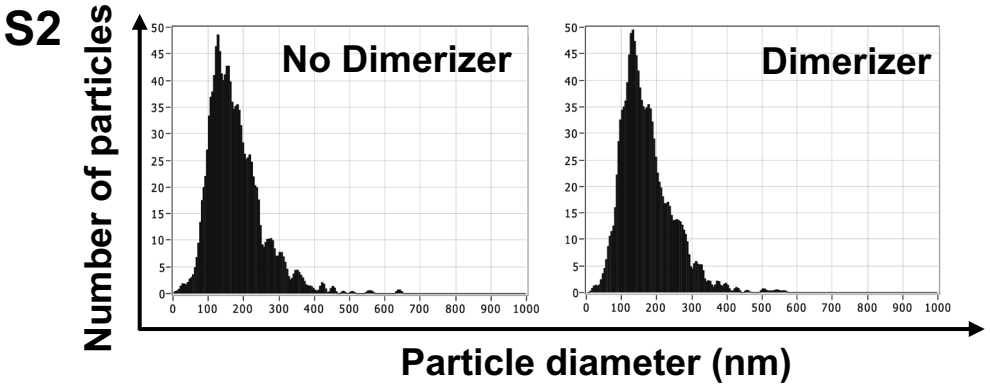

Figure S2. ***NanoTracking Analysis.*** Size distribution of EVs produced in presence (“Dimerizer”) or absence (“No Dimerizer”) of A/C dimerizer drug.

S3

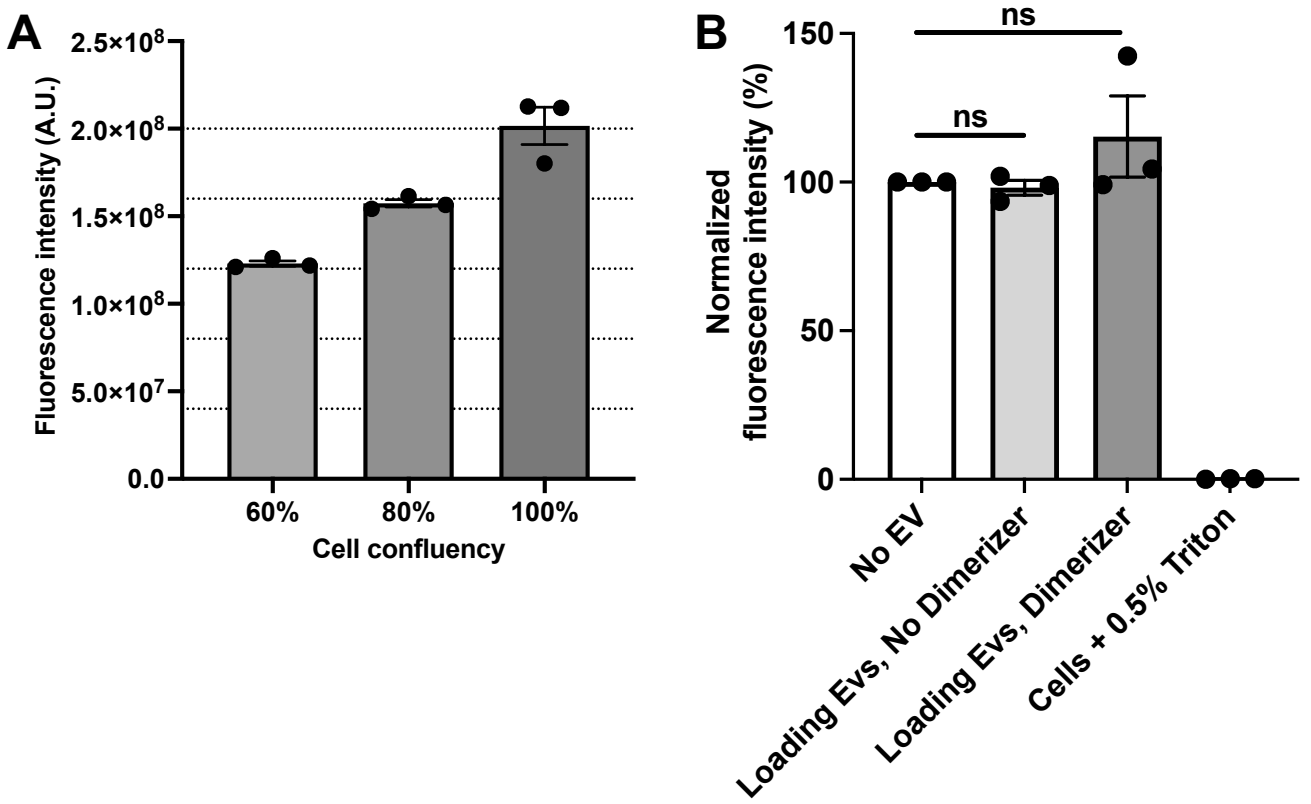

Figure S3. ***Alamar blue assay.*** (A) Cells were seeded at three different densities, and incubated in Alamar blue reagent for 2h the next day. This experiment shows the cell-dependency and proportionality of the assay. A.U., arbitrary unit. Each point is a technical replicate. Mean represented ± SEM. (B) HeLa WT were incubated during 24h with or without EVs harboring both $FKBP_2$-RFP-CD63 and FRB-NLuc-HA ("Loading EVs") produced in presence ("Dimerizer") or absence (No Dimerizer") of A/C dimerizer, or treated during 2h with 0.5% Triton. An Alamar blue assay was performed. Each dot represent the mean of three technical replicates. Mean ± SEM represented. For statistical analysis, a Kruskal-Wallis test was performed with $p \geq 0.05$ non significant (ns).

S4

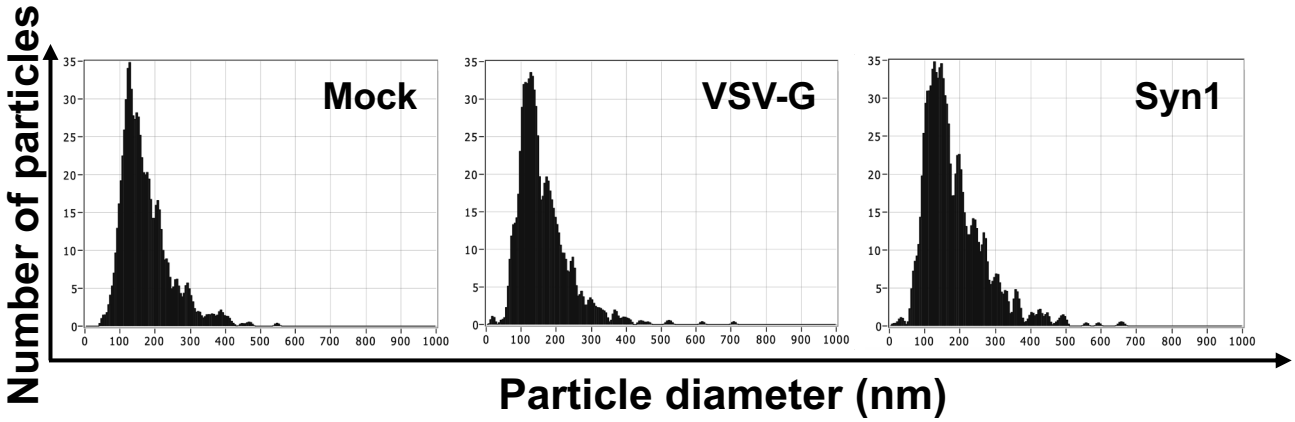

Figure S4. ***NanoTracking Analysis.*** Size distribution of EVs carrying mCherry ("Mock"), or harboring VSV-G or Syn1.

S5

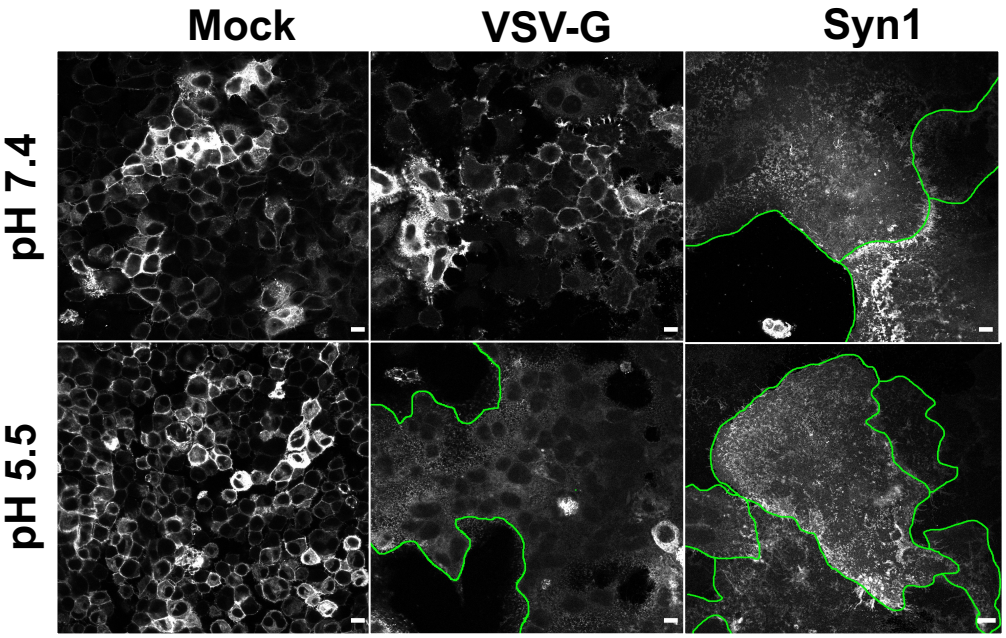

Figure S5. ***Fusion assay.*** Stable CD8-GFP^{+} HeLa were transfected with an empty vector ("Mock"), or a plasmid encoding for VSV-G or Syn1. Then, they were incubated ("pH 5.5") or not ("pH 7.4") within a fusion buffer, and imaged by confocal microscopy. Representative of two independent experiments. Green line, syncytia membrane. Scale bar, 10μm.

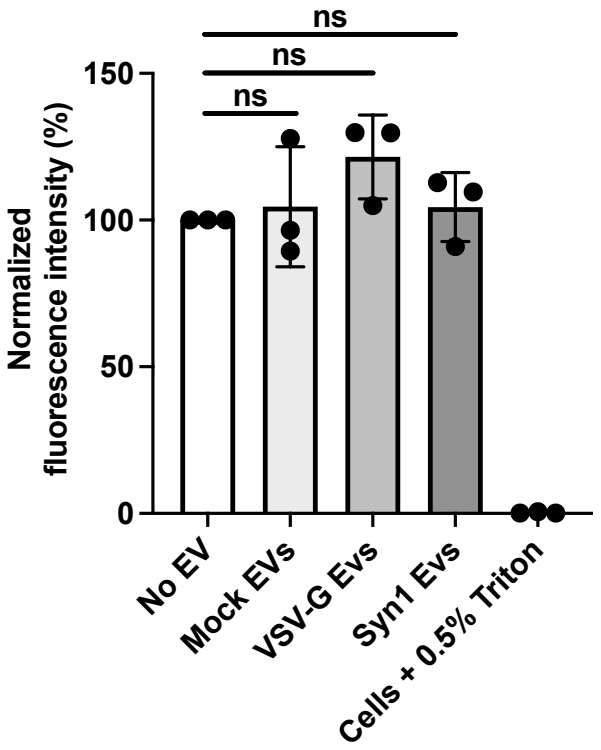

Figure S6. ***Alamar blue assay.*** HeLa WT were incubated during 24h with or without EVs carrying mCherry ("Mock"), or harboring VSV-G or Syn1, or treated during 2h with 0.5% Triton. An Alamar blue assay was performed. Each dot represent the mean of three technical replicates. Mean ± SEM represented. For statistical analysis, a Kruskal-Wallis test was performed with $p \geq 0.05$ non significant (ns).

S7

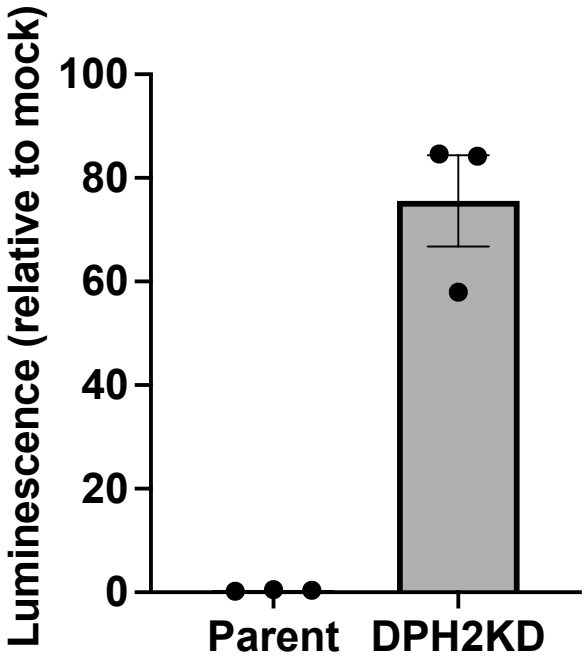

Figure S7. WT or DPH2KD cells were co-transfected with FRB-DTA and plasmid encoding NLuc-Hsp70. NLuc-activity was measured 24 hours *post*-transfection to assess protein synthesis. Each point represent an independent experiment. Mean ± SEM represented.

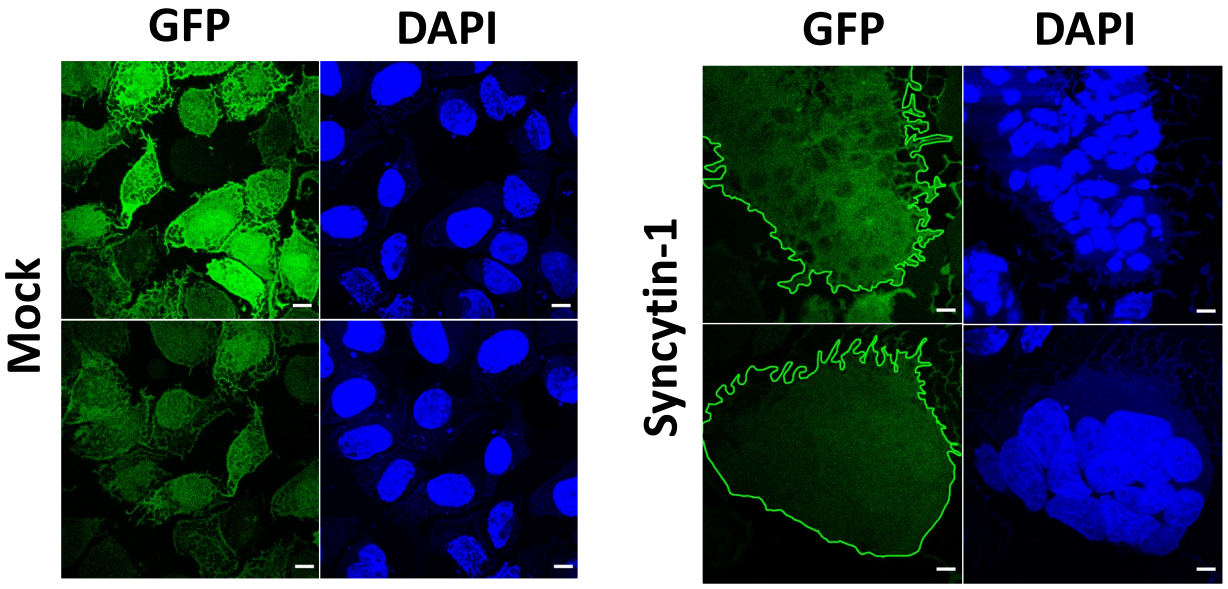

Figure S8. ***Fusion assay.*** GFP-PEST^{+} HT1080 cells transfected with an empty vector ("Mock") or encoding for Syncytin-1 and imaged by confocal microscopy. Green line, syncytia membrane. Scale bar, 10μm.

S9

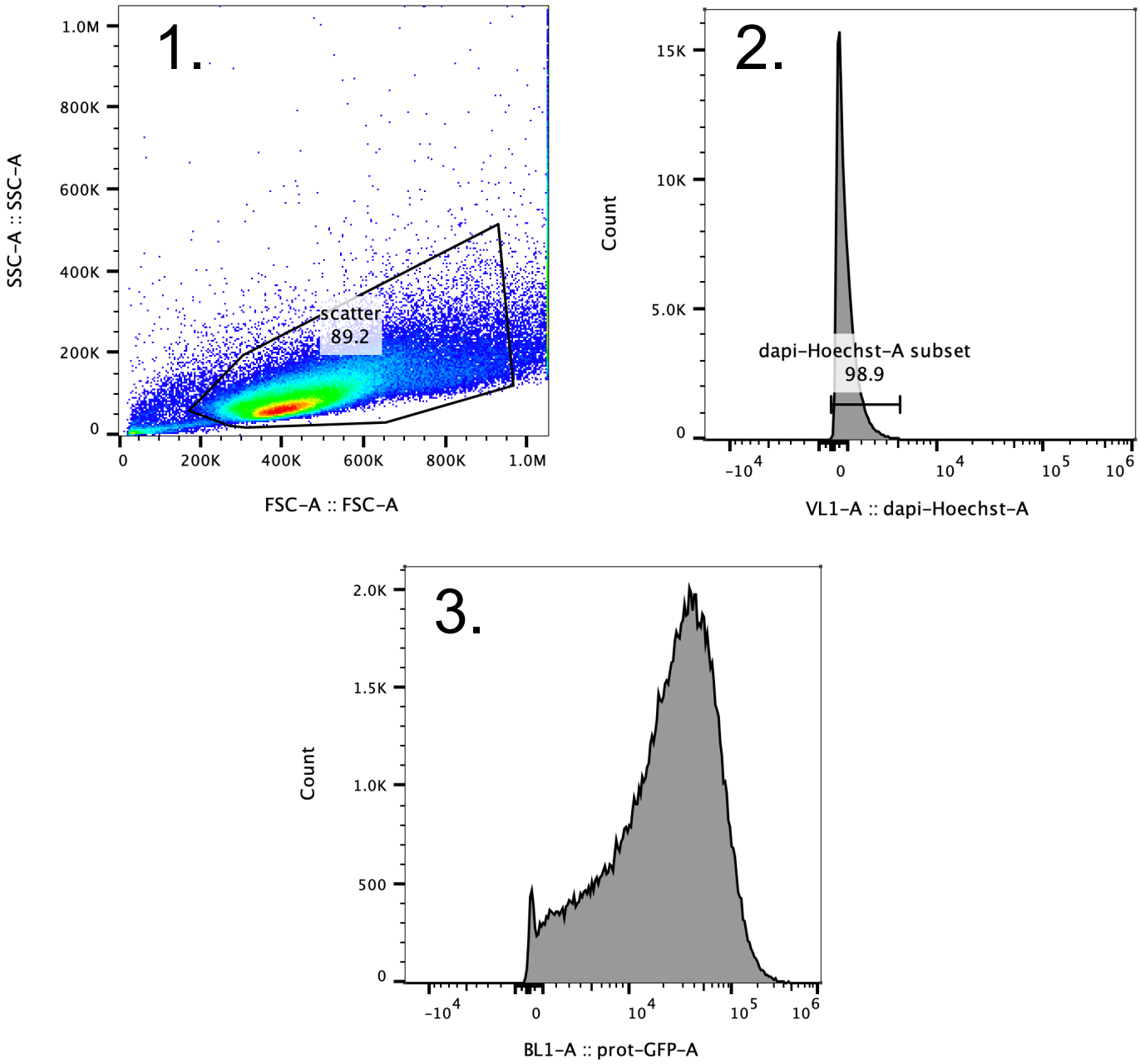

Figure S9. FACS gating strategy used in the killing assay. (1) First, the cell population was defined by a scatter gate on the SSC-A/FSC-A plot. (2) Then, a DAPI-negative gate was applied to obtain the GFP fluorescence plot in (3).